



**Branch junction constriction and hydraulic limitation in
two species in the Cape Proteaceae: A mechanism
explaining the trade-off between longevity and degree of
ramification in the Cape Proteaceae.**



Research in Ecology

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ABSTRACT

The purpose of this study was to establish if hydraulic limitation and branch junction constriction explain the trade-off between longevity and increased ramification in species of the Cape Proteaceae. This was done by establishing if branch nodes are regions of greater resistance to xylem sap flow in the study species; attempting to quantify nodal resistance for a plant as a whole; and by determining if there is any evidence for hydraulic limitation or compensation in *Leucadendron laureolum* (Lam.) Fourc. and *Leucospermum oleifolium* (Berg.) R. Br. individuals.

The extent of branch junction constriction was established by comparing the ratio of the xylem sap flow rates of adjacent branch segments of equal length. The ratio of a proximal internodal to a distal internodal segment was compared against the ratio of a proximal internodal segment to a distal node. Branch nodes were found to create significant resistance to xylem sap flow rates in both species ($p < .005$ for *L. laureolum*, and $p < .05$ for *L. oleifolium*).

Total nodal resistance in *L. laureolum* was modelled as equivalent extra branch length, and as loss of sap flow rate. Equivalent extra branch length increased exponentially with increased branching order, and increased much faster in more ramified plants. Loss of flow rate increased linearly with increased branching order and was unaffected by differences in ramification. This was because the nodes in more ramified plants had smaller basal sapwood areas, and the resistance imposed by nodes decreased with decreased basal sapwood area. As more ramified plants tend to branch earlier, and/or with greater temporal frequency, they will still accumulate greater nodal hydraulic resistance faster than less ramified plants, and thus may be limited to smaller size and younger maximum age.

Branch specific conductivities (k_s), leaf specific conductivities (LSC) and transpiration rates were investigated in higher (11 nodes) and lower (10 nodes) order branches of *L. laureolum*. All were found to be significantly lower in higher order branches (11 nodes), which can be related to greater nodal resistance to sap flow limiting the amount of water available to the area of developing xylem, causing the vessels to have narrower diameters. There was no significant difference in leaf to sapwood area ratios (A_l/A_s) between the two branching orders. Previous studies have suggested that changes in A_l/A_s ratios occur to compensate for hydraulic limitation. Reductions in A_l/A_s in plants with greater hydraulic restriction allow LSC values, and thus transpiration rates, to stay as high as plants with less hydraulic restriction. As there was no such compensation, LSC values, and thus transpiration rates, were much lower in branches of higher order. Transpiration rate is a good indication of rates of photosynthesis. Lower transpiration rate in higher order branches is thus a good indication of hydraulic limitation on rates of photosynthesis with increased branching order.

Hydraulic resistance in nodes thus imposes an important limitation on size and/or age of *L. laureolum* individuals, and explains the apparent trade-off between longevity and degree of ramification in the Cape Proteaceae.

1) INTRODUCTION

The Cape Floristic Region (CFR) of South Africa contains roughly 9000 species of vascular plants, of which approximately 69% are endemic (Goldblatt and Manning 2000). The region makes up less than 0.5% of Africa's land area, but contains almost 20% of all the vascular plant species found on the continent (Goldblatt and Manning 2000). This amazing diversity is comparable to the most diverse equatorial regions, with the large contribution of a few plant families to the overall species richness being reminiscent of island floras (Linder 2003). Many studies have identified factors that have contributed to the massive species richness in the region. Linder (2003) divided these factors into those that limit gene flow and those that drive disruptive selection. Geographic isolation, inter-specific sterility barriers, and reproductive isolation through pollinator specificity (Johnson *et al.* 1998), or temporal separation of flowering times (Linder 2001), have been identified as important factors limiting gene flow between populations and species. Edaphic specialization, climatic specialization (Verboom *et al.* 2003), adaptation to disturbance by fire (le Maitre and Midgley 1992, Bond and Midgley 1995, Linder 2001) and microhabitat specialization (Richards *et al.* 1995) are all believed to be factors driving disruptive selection in the CFR.

Fires are an important environmental disturbance in the CFR and most species in the region have evolved some way of surviving or regenerating after fire (le Maitre and Midgley 1992, Linder 2003). Many species have evolved a dependency on disturbance by fire, and risk extinction if the vegetation doesn't burn between every 5 and 50 years (Le Maitre and Midgley 1992). The family Proteaceae makes a significant contribution to the diversity of the CFR with 330 species in 14 genera found in the region (Rebelo 1995). The Proteaceae are arguably the most conspicuous of the plant families in the CFR forming the dominant overstorey in most communities (Cowling and Holmes 1992). The CFR is a major centre of diversity for the family Proteaceae with ten genera, and approximately one fifth of all species, endemic to the region. Fire survival strategies are thought to have been a particularly important factor driving disruptive selection in the Cape Proteaceae, promoting speciation and species coexistence through niche differentiation (Bond and Midgley 1995). Fire survival strategy is affected by a number of interrelated traits (Bond and Midgley 1995, Midgley and Bond 1989, Midgley and Kruger 2000), which also affect a number of other ecological and physiological attributes of the plant. "Corner's Rules" relate the degree of ramification of a plant to branch diameter, and branch diameter to leaf or inflorescence size. Midgley and Bond (1989) highlighted that as leaf and inflorescence size are related to branch size, they should be related to each other, suggesting a trade-off between the reproductive ecology and

physiological processes of a species. Midgley and Kruger (2000) found that plant size was negatively related to the degree of ramification in the Cape Proteaceae, and thus postulated that appendage size should be positively correlated with plant size. In addition to these allometric relationships species of the Cape Proteaceae have terminal inflorescences, and thus the number of inflorescences they can support is directly related to their degree of ramification. The relationships between these traits thus represent major trade-offs as they determine various ecological and physiological attributes such as fire survival strategy, relative growth rate and maximum size among others (Midgley and Bond 1989, Midgley and Kruger 2000, Westoby *et al.* 2002, Wright *et al.* 2004). Plants with fine foliage and thin branches have greater lateral canopy spread and higher surface to volume ratios, allowing them to produce more seed, than plants with larger leaves and thicker branches. This results in fine-leaved plants having faster relative growth rates, but also makes them more flammable (Bond and Midgley 1995). As fine-leaved species have higher relative growth rates and branch more frequently, they reach reproductive maturity faster than large-leaved species (Bond and Midgley 1995). Highly ramified, fine-leaved “torches” are thus thought to promote and benefit from frequent and intense fires, whereas less ramified, large-leaved “damps” are thought to suppress fires, benefiting from longer inter fire intervals or low intensity fires (Bond and Midgley 1995).

In their study on senescence in the Cape Proteaceae, Midgley and Kruger (2000) found that highly ramified species are generally smaller in stature, and senesce younger, than less ramified species within a site. They found a negative correlation between maximum lifetime height and the ratio of an index of canopy spread to basal diameter, and suggested that death was caused by mechanical failure as the result of a top-heavy architecture. This suggests that the selective pressure for “torch” fire survival strategies is so pervasive that longevity is sacrificed to ensure a more effective “torch” strategy. This is not surprising because if individuals were killed in regular, frequent fires there would be little selection for longevity. Taller, less ramified “damps” on the other hand may survive fires of lower intensity, and are thus likely to be under stronger selection for longer life spans.

Senescence involves the complex of aging processes that eventually lead to death (Lawrence 1993). Midgley and Kruger’s (2000) hypothesis that death is caused by mechanical failure as the result of a top-heavy architecture may not fully explain senescence in the Cape Proteaceae. A mechanism that could relate increased ramification to true senescence is increased hydraulic limitation caused by branch junction constriction. The hydraulic limitation hypothesis (Ryan and

Yoder 1997) proposes that increased hydraulic resistance increases vulnerability to drought stress and causes lower leaf photosynthesis, ultimately limiting plant size. Plants may increase their hydraulic resistance, through growth and increased ramification, to a critical maximum for a given soil water potential (Ryan and Yoder 1997, Koch *et al.* 2004). A subsequent drop in the soil water potential would result in permanent wilting of leaves because their water potentials would drop causing stomata to close and vessels to embolise (Tyree and Zimmermann 2002). If the soil water potential stays low for an extended period of time the stomata will remain closed, preventing photosynthesis taking place, eventually resulting in the death of the plant. Branch junctions have been reported to cause hydraulic restrictions in a number of North Temperate tree species (Zimmermann 1978, Ewers and Zimmermann 1983, Tyree *et al.* 1991, Tyree and Alexander 1993, and Schulte and Brooks 2003).

Most species in the Cape Proteaceae have terminal inflorescences and typically add a new branching order each year after each flowering event, resulting in a dense, spherical, sympodial architecture with no main axis. If nodes create hydraulic resistance in the Cape Proteaceae then the yearly addition of nodes will rapidly accumulate a large amount of resistance. Plants with a sympodial architecture that produce greater numbers of branches per node create more nodes per branching order than plants that produce fewer branches per node. Thus more ramified species have a greater number of nodes than less ramified species for any particular branching order (\approx age in Cape Proteaceae). Should height and growth in Cape Proteaceae be hydraulically limited, and should nodes create hydraulic restrictions, then increased numbers of nodes would create greater hydraulic resistance thereby limiting maximum size and life span of more ramified species sooner than less ramified species.

Hubbard *et al.* (2002) suggested that plants mitigate the effects of increased hydraulic resistance by decreasing leaf area to sapwood area ratios (A_l/A_s) with increased plant height. Essentially they argue that if there is more sapwood supplying each unit of leaf area then the water potentials do not become so negative as to cause stomatal closure or embolism. This would allow the plant to grow larger before reaching a critical maximum hydraulic resistance. It does, however, mean that as a plant increases in size the photosynthetic area to live biomass ratio decreases, and thus production to respiration rates decrease. In a hydraulically limited species with sympodial architecture an older plant with a higher order of branching, and thus more nodal resistance, should have lower A_l/A_s ratios than plants with fewer branching orders.

This study is an attempt to establish if nodes are regions of greater resistance to xylem sap flow in two species in the Cape Proteaceae; to quantify this resistance per node, and for a plant as a whole; and to determine if there is any evidence for hydraulic limitation or compensation in the study species. The primary objective is to establish if hydraulic limitation and branch junction constriction can explain the relationship between ramification and longevity in species of the Cape Proteaceae.

2) MATERIALS AND METHODS

i) Data collection:

a) Study site

The study site was located on “Mountain Rose” farm in the Palmiet river valley (34°19'35.3"S; 18°58'22.3"E), in the Western Cape Province of South Africa. The site falls within the winter rainfall region of the Western Cape Province of South Africa, and receives approximately 940mm rainfall spread over 120 days every year (Boucher 1978). Soils were sandy, with high quartz content, derived from the Peninsula sandstone formation. Specimens were sampled from a stand of co-occurring *Leucadendron laureolum* (Lam.) Fourc. and *Leucospermum oleifolium* (Berg.) R. Br. individuals to ensure that the plants had grown in similar moisture conditions. The stand had a dense restionaceous understorey, with the study species' forming the greater part of the overstorey.

b) Study specimens

Leucospermum oleifolium (Berg.) R. Br. is a monoecious species that occurs between altitudes of 0 and 1000 metres from the Kogelberg to the Hottentots Holland and Franschoek mountains (Vogts 1982). They are typically sunbird or bee pollinated and release seeds that have fatty bodies called eliasomes that facilitate dispersal by ants. Individuals do not have the ability to resprout, and typically have compact, rounded canopies arising from a single stem (Rebelo 1995). *Leucadendron laureolum* (Lam.) Fourc. is a relatively common dioecious species along the south coast of South Africa, occurring anywhere from sea level to 1000 metres in altitude (Vogts 1982). Pollen dispersal is achieved by a combination of wind and generalist insect pollinators. Wind

dispersed seeds are retained in cones on female individuals and are released after the plant is killed by fire (serotiny). Male individuals do not retain their cones. Individuals are single stemmed and do not have the ability to resprout (Rebello 1995).

L. oleifolium and male *L. laureolum* individuals were chosen to avoid possible complicating factors such as the retention of cones, or the ability to resprout. Serotinous cones dehydrate and open to release their seeds when they are removed from a plant, or the plant to which they are attached is killed (Vogts 1982), suggesting that they incur a hydraulic cost to the plant. As the cones are located at branch nodes they would most likely confound the findings of this study. Resprouting species were avoided to avoid any unforeseen complicating factors. Resprouting individuals are often multistemmed, and occasionally resprout from underground storage organs after their above ground structures have senesced or been killed. Branches were sampled from three *L. oleifolium* individuals, and three larger (11 branching orders on plants) and three smaller (10 branching orders on plants) male *L. laureolum* individuals. This allowed comparison of the degree of nodal resistance between species in two major genera of the Cape Proteaceae, and allowed the investigation of changes in the degree of hydraulic limitation experienced by plants of different branching order.

c) Measurement of hydraulic conductivity (k_h)

Hydraulic conductivity (k_h , in $\text{kg m MPa}^{-1} \text{s}^{-1}$) of stem segments was calculated, from measurements of the mass of water flowing through an embolism free segment per unit time at a constant pressure, using the equation:

$$k_h = F * (L / \Delta P) \quad (1)$$

where F = flow rate (kg s^{-1}), L = segment length (m) and ΔP = pressure drop (MPa).

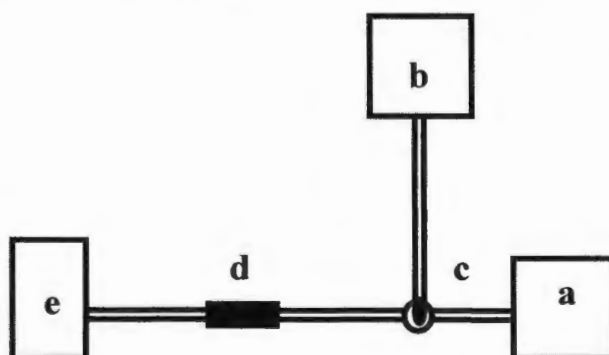


Figure 1: Schematic diagram of the modified Sperry apparatus used to measure hydraulic conductivity. High pressure reservoir (a) and low pressure reservoir (b) were connected to the segment of branch (d) by PVC tubing. A three-way stopcock (c) controlled which reservoir was in use. The amount of solution flowing through the branch segment was measured on a four-point balance (e) as it collected in a beaker.

Flow rates were measured using a modified Sperry apparatus (Sperry *et al.* 1988) shown in figure 1. A filtered (0.1 μm membrane filter) 10 mM KCl solution with degassed, de-ionized water was used to prevent clogging of the xylem vessels with

particles or air bubbles, and to avoid the unexplained decline in measured flow rates over

time reported in a number of previous studies (Zimmermann 1978, Sperry *et al.* 1988, van Ieperen *et al.* 2000). Segments were cut under water, and the ends shaved using a sharp Stanley knife. Initial water flow rate for each segment was measured at 7.5 kPa from a low-pressure reservoir by reading off the mass (in grams) of the collecting beaker on a four point balance every 30 seconds for 2.5 minutes. The segment was then flushed for 20 minutes at 150 kPa, to remove emboli, and the flow rate re-measured. This procedure was repeated until the flow rate stabilized, presumably because all emboli had been removed, giving the maximum flow rate for each segment.

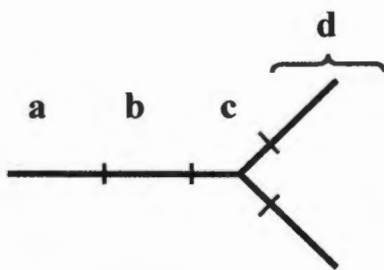


Figure 2: Diagrammatic representation of full segment. Segments a and b are proximal to the nodal segment c. Segment d consists of all segments distal to the node.

Flow rates were measured on stem segments as shown in figure 2. Some segments had three branches emerging from the node, and others had two. The full segments (a-d) were 20 cm long, comprising of four 5 cm segments, for the *L. laureolum* and 16 cm long, comprising of four 4 cm segments, for the *L. oleifolium* specimens. Flow rates for the full segments were measured initially, and then re-measured as each of the smaller segments were removed from the base (figure 2). The flow rates through the individual smaller segments were measured separately. The branch segments distal to the node were considered one segment (d), and the flow rate through the combined segments measured simultaneously.

d) Leaf and sapwood area measurements

The numbers of leaves distal to each stem segment were counted, and leaf area was determined using a LI-COR, LI3100 leaf area meter. The sapwood in each stem segment was stained by connecting the segments to the base of a vertical two metre piece of clear PVC piping filled with a 0.5% safrinin dye, 10mM KCl solution with degassed, de-ionized filtered (0.45 μm membrane filter) water, allowing for gravity flow of the dye through the segment for more than a minute. Cross sections of the stained segments were photographed and analyzed using the *Sigmascan Pro 5.1* image analyzing software package to determine sapwood areas. Leaf area to sapwood area ratios (A_l/A_s) were calculated by dividing the leaf area by the sapwood area ($\text{m}^2 \text{m}^{-2}$). Specific hydraulic conductivity (k_s , $\text{kg Mpa}^{-1} \text{s}^{-1} \text{m}^{-1}$) and leaf specific conductivity (LSC, $\text{kg Mpa}^{-1} \text{s}^{-1} \text{m}^{-1}$) were calculated by dividing the hydraulic conductance (k_h) by the sapwood area (m^2) and the leaf area (m^2) respectively.

ii) Data analyses:

a) Xylem vessel length

It is generally believed that hydraulic conductivity can only be measured on segments longer than the longest xylem vessels (Zimmermann 1978, Sperry *et al.* 1988, Ewers and Fisher 1989), as most of the resistance to water flow is thought to occur in the perforation plates. Segments cut shorter than the longest xylem vessels may contain vessels with no perforation plates through which water can pass unhindered. This results in the shorter segments having much lower resistance per unit length than the segments longer than the longest xylem vessels.

A rough measure of maximum vessel length was gained by pumping nitrogen gas through the base of the stem at 150 kPa while underwater, and trimming the end of the stem back until bubbles emerged from the cut end (Ewers and Fisher 1989).

The maximum length segments could be cut in this study was limited by the lengths of the internodes of the study species, and the sampling design shown in figure 2. Chui and Ewers (1993) suggested that the importance of taking xylem vessel length into account when measuring hydraulic conductivity depends on the question being asked. In order to justify the use of segments shorter than the longest vessel lengths, the resistance of whole segments (eg. a to d in figure 2), were compared against the sum of the individually measured resistances of the component parts ($a + b + c + d$). This allowed four data points for each node (a vs. a, ab vs. $a + b$, etc.). If the slope of the relationship is one (i.e. there is a direct positive relationship between the resistance of complete segments and the sum of the resistances of their component parts) then perforation plates have no observable effect on the hydraulic conductivity of the stem segments, and xylem vessel length can be ignored when segments are cut. Similarly, if the relationship is linear then the ratios used to compare flow rates of nodal and internodal segments (discussed below) remain true.

b) Identifying and quantifying hydraulic restriction in nodes

Branch length, branch diameter and/or sapwood area or vessel number, and vessel diameters are all important in facilitating water transport (Zimmermann 1978). Any differences in these factors between compared branched and unbranched segments would confound results. To

overcome this problem the relative increase in resistance created by successive stem segments of equal length (figure 2) were compared. The hydraulic conductivity of equal length segments should decrease as one moves distally along a branch as the branch tapers and the number and diameter of xylem vessels decrease (Tyree and Ewers 1991, Koch *et al.* 2004). Conductivities would thus be expected to decrease from segment to segment when compared directly, even in the absence of branch nodes. The ratio of the flow rates of one straight segment to another straight segment (a and b in figure 2) was compared with the ratio of the flow rates of a straight segment to a branched segment (b and c) so as to overcome inherent differences in conductivities. The results were compared using t-tests.

It is very difficult to separate the inherent increase in resistance from segment to segment, moving distally along a branch, from the extra resistance created by a hydraulic constriction. A graph of the mean change in conductivity from segment to segment, for both species, is shown in figure 4. The differences in conductivities in segments from different nodes were standardized by dividing the conductivity of each segment in each node by the mean conductivity for that node.

The drop in flow rate caused by each node was estimated using the equation:

$$R_{\text{node}} = (F_b - F_c) - (F_c - F_d) \quad \text{where } (F_c - F_d) > 0 \quad (2)$$

where F_b , F_c and F_d are the flow rates through segments b, c and d respectively, and are expressed as kg s^{-1} . $(F_b - F_c)$ represents the drop in flow rate caused by segment c (the segment containing the node), and $(F_c - F_d)$ represents the standard expected drop. Where $(F_c - F_d)$ was negative it was replaced with zero. The drop $(F_a - F_b)$ was not used to calculate the standard drop, as the values were often negative. The proportion of segment c's resistance found in the node was calculated by dividing R_{node} by $(F_b - F_c)$. The mean, standard error and standard deviations of the drop in flow rate caused by the nodes, and the proportion of segment c's resistance found in the node, were displayed as box and whisker plots. The values were also plotted against the basal sapwood area of each node (cm^2).

c) Investigating the effects of hydraulic limitation, and mechanisms of compensation, in different sized *L. laureolum* individuals

A_l/A_s ratios, specific conductivity, leaf specific conductivity, leaf area, sapwood area and hydraulic conductivity values were compared between 10th and 11th order branches in *L. laureolum* individuals using t-tests. All three values were measured the same number of nodes back from the

branch tips on every individual to avoid any possible bias in leaf areas created by nodal resistance that was unaccounted for.

Transpiration rates at mid-day were measured on plants with 9, 10 and 11 branching orders using a Li-Cor Steady state nul balance Porometer LI-1600 and an IRGA ADC LCA4. The mean values, of three readings per plant, for three individuals of each branching order were taken. Readings were taken on leaves with the same number of nodes between them and the base of the plant, for each plant, to avoid intra-canopy variation. Transpiration rates were compared using Mann-Whitney U-tests.

3) RESULTS

a) Xylem vessel lengths

The longest vessel lengths measured were 500 ± 10 and 585 ± 10 mm for *L. laureolum* and *L. oleifolium* respectively. These were far longer than the segments for which hydraulic conductivity were measured.

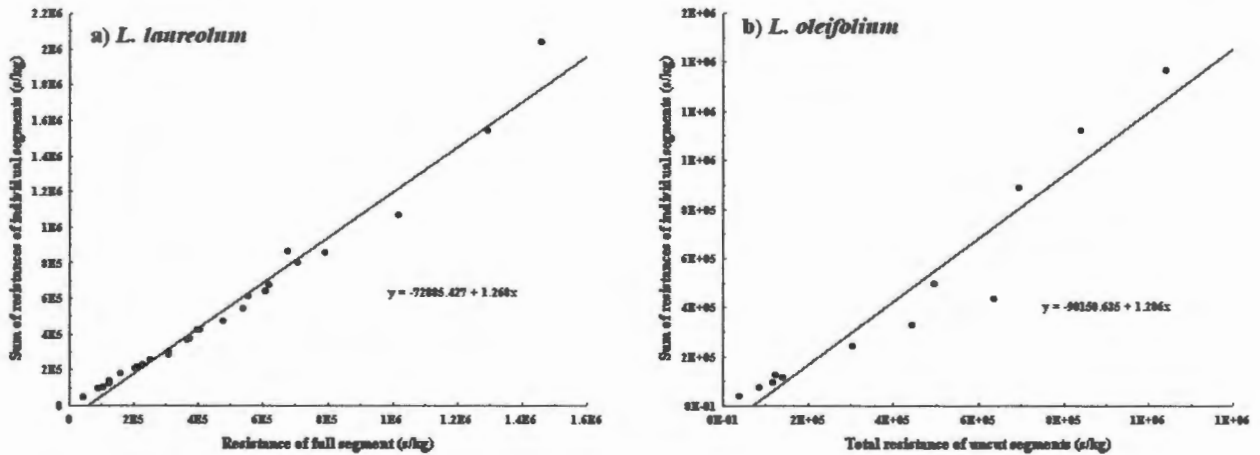


Figure 3: The resistance of uncut segments plotted against the sum of the resistances of their component parts for a) *L. laureolum* ($R^2 = 0.99$, $B = 1.17$, $p < 0.0001$, $N = 28$) and b) *L. oleifolium* ($R^2 = 0.96$, $B = 1.15$, $p < 0.0001$, $N = 12$). Regression analyses were constrained to pass through the origin.

The relationship between the resistance of the complete segments, and the sum of the resistances of the component smaller segments, is strongly linear when the y-intercept was estimated ($R^2 = 0.98$, $N = 28$, $p < 0.0001$ and $R^2 = 0.92$, $N = 12$, $p < 0.0001$ for *L. laureolum* and *L. oleifolium* respectively; figure 3). The slopes of the regression equations were slightly larger than one ($B = 1.27$ and 1.29 for *L. laureolum* and *L. oleifolium* respectively) implying that the

component smaller segments created greater resistance to water flow than the complete segments. The coefficients of variation, and the slopes of the regression equations, were closer to one when the regression line was forced to pass through the origin ($R^2 = 0.99$, $B = 1.17$ and $R^2 = 0.96$, $B = 1.15$ for *L. laureolum* and *L. oleifolium* respectively), indicating a very nearly direct relationship.

Table 1. Summary statistics of the correlation co-efficients and slopes for the regression of the sum of resistances of individual segments against the total resistance of uncut segments for seven *L. laureolum* and three *L. oleifolium* nodes.

	<i>L. laureolum</i>		<i>L. oleifolium</i>	
	r^2	B	r^2	B
Mean	0.986	1.183	0.994	0.973
Minimum	0.954	0.959	0.987	0.603
Maximum	1.000	1.551	0.998	1.587
Standard deviation	0.021	0.210	0.006	0.535

Note: N = 4 for all correlations
All correlations significant at the $p < .05$ level

When plotted separately for each node the sum of the resistances of the separate segments was much less than the resistances of the uncut segments for only one of the *L. oleifolium* nodes (table 1). Strong linear relationships were evident in all nodes.

b) Identifying and quantifying hydraulic constriction in nodes

Both species showed a large drop in conductivity from the first basal segment (b) to the nodal segment (c) (figure 4). There was also a slight increase in conductivity from the second (a) to first (b) basal segments. The drop in conductivity from segments c to d is as expected as one moves distally along a branch (Tyree and Ewers 1991, Koch *et al.* 2004).

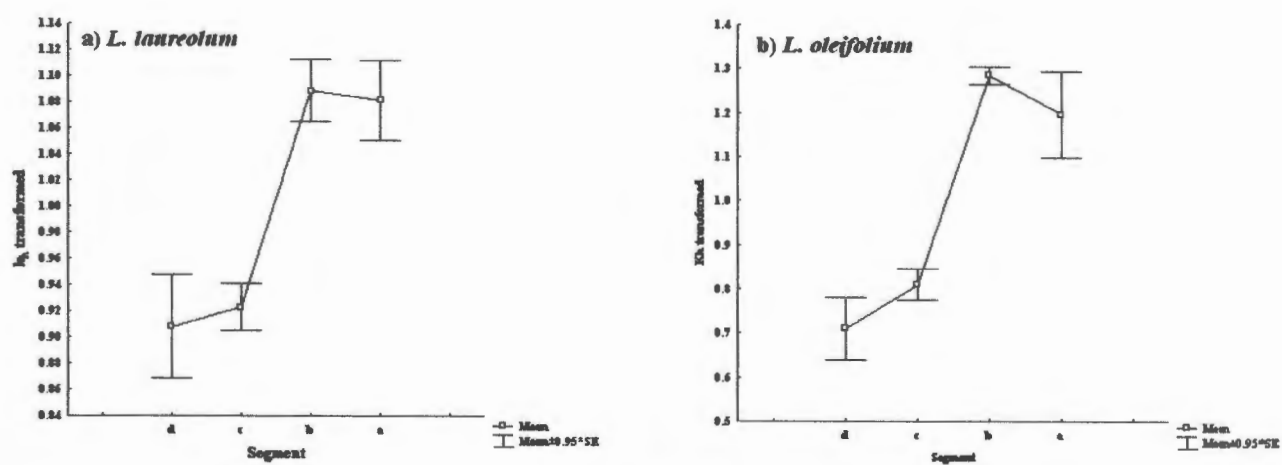


Figure 4: The mean transformed k_h values for the segments in each position for a) *L. laureolum* (N = 7) and b) *L. oleifolium* (N = 3). Conductivity (k_h) values were transformed by dividing by the mean value of the four individual component segments for each complete segment.

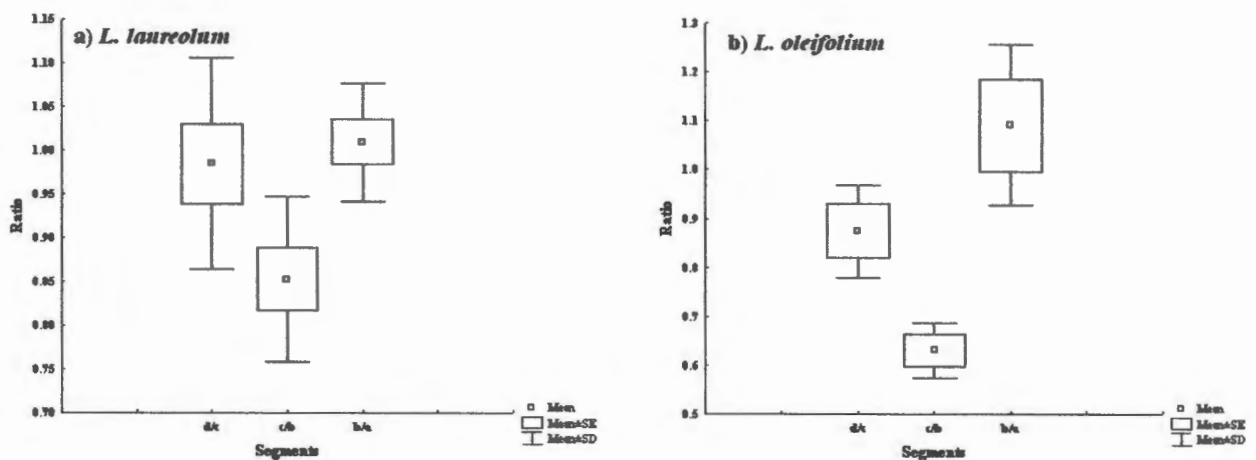


Figure 5: The ratios of the k_h values of each segment divided by the segment immediately basal to it for a) *L. laureolum* (N = 7) and b) *L. oleifolium* (N = 3).

The means, standard errors and standard deviations of the ratios of the conductivities of each segment divided by the segment immediately basal to it are shown for both species in figure 5. This indicates the extent to which conductivity drops from one segment to the next. The c/b ratio is significantly smaller than the b/a and d/c ratios in both species ($p < 0.05$), indicating that the drop in conductivity from segment b to segment c is significantly greater than the drop from segment a to b or segment c to d (table 2). Levene, and Brown-Forsythe tests of the data in all t-tests performed were not significant, indicating that the assumption of homogenous variances, required for t-tests to be valid (Legendre and Legendre 1998), is satisfied.

Table 2. T-tests for differences between the ratios of the conductivities of each segment divided by the segment immediately basal to them (shown in figure 5). Levene and Brown-Forsythe tests for homogeneity of variance are also reported.

Ratios	T-value	df	p	Levene F	df	p (Levene)	Brown-Forsythe F	df	p (Brown-Forsythe)
<i>L. laureolum</i>									
d/c vs c/b	2.280	12	*	0.032	12	NS	0.061	12	NS
c/b vs b/a	-3.574	12	***	2.312	12	NS	1.489	12	NS
d/c vs b/a	-0.473	12	NS	1.451	12	NS	1.477	12	NS
<i>L. oleifolium</i>									
d/c vs c/b	3.818	4	*	0.657	4	NS	0.408	4	NS
c/b vs b/a	-4.564	4	*	3.428	4	NS	1.026	4	NS
d/c vs b/a	-1.975	4	NS	1.248	4	NS	0.364	4	NS

NS = $> .05$, * = $< .05$, ** = $< .01$, *** = $< .005$

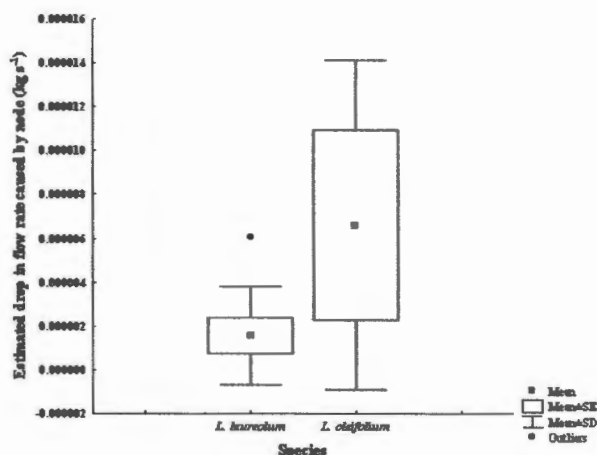


Figure 6: The estimated drop in flow rate caused by nodes in *L. laureolum* (N = 7) and *L. oleifolium* (N = 3)

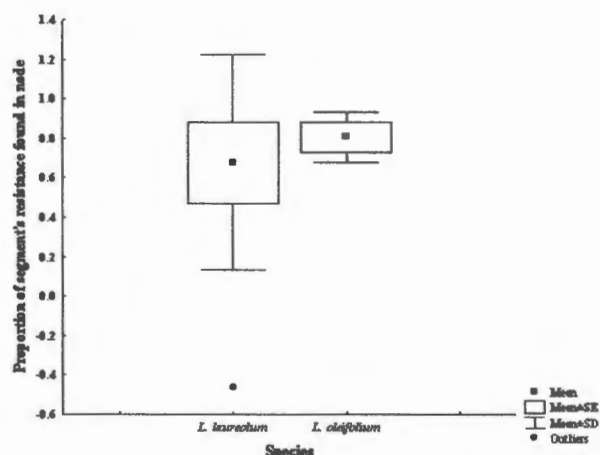


Figure 7: The proportion of segment c's resistance found in the node for *L. laureolum* (N = 7) and *L. oleifolium* (N = 3). The difference between them is not significant (Kruskal-Wallis H)

The drop in flow rate caused by nodes was larger in *L. oleifolium* than in *L. laureolum* (figure 6). When the drop was expressed as a proportion of the total drop across the nodal segments (figure 7) there was no significant difference between the two species (Kruskal-Wallis H (1, 10) = 0.33, $p > 0.05$). A plot of the drop in flow rate caused by the node against the basal sapwood area of each node for *L. laureolum* shows that the amount of extra resistance in the node increases with increased basal sapwood area ($R^2 = 0.82$, $p < 0.005$, N = 7; figure 8). A similar plot of the proportions of the total drop across the nodal segments shows no clear trend ($R^2 = 0.10$, $p > 0.05$, N = 7; figure 9).

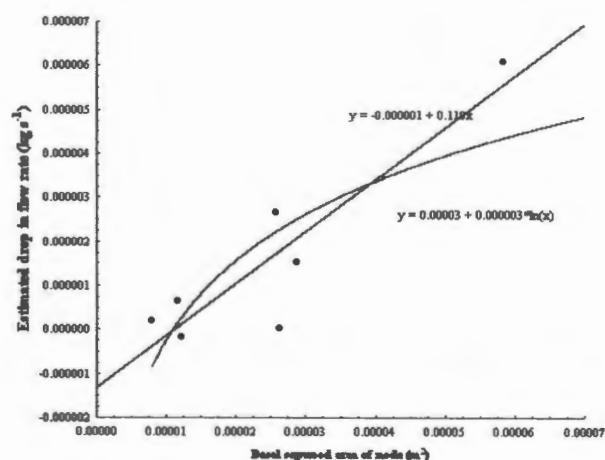


Figure 8: The estimated drop in flow rate caused by each node plotted against the basal sapwood area of the node in *L. laureolum* (linear regression: $R^2 = 0.82$, $p < 0.005$, N = 7; logarithmic regression: $R^2 = 0.64$, $p < 0.05$, N = 7).

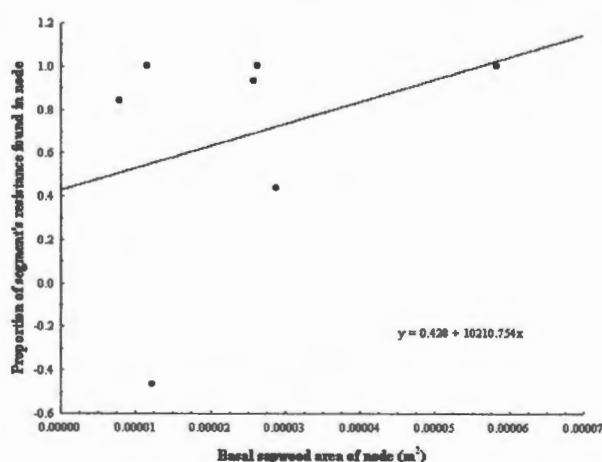


Figure 9: The proportion of segment c's resistance found in each node plotted against the basal sapwood area of the node for *L. laureolum* ($R^2 = 0.10$, $p > 0.05$, N = 7)

c) Investigating the effects of hydraulic limitation, and mechanisms of compensation, in different sized *L. laureolum* individuals

There was no significant difference between the A_l/A_s ratios of the branches of each order (t-value = 0.07, df = 6, $p > 0.05$; table 3). This means that the cross sectional area of sapwood supplying each unit of leaf area is similar in branches of both orders.

Table 3. T-tests for differences in A_l/A_s ratios, sapwood areas, k_h , and leaf areas between the smaller and larger *L. laureolum* individuals. Levene and Brown-Forsythe tests for homogeneity of variance are also reported.

Measure	T-value	df	p	Levene F	df	p (Levene)	Brown-Forsythe F	df	p (Brown-Forsythe)
A_l/A_s (m ² /cm ²)	0.072	6	NS	0.012	6	NS	0.012	6	NS
Sapwood area (cm ²)	0.400	6	NS	1.814	6	NS	0.163	6	NS
k_h	-0.046	6	NS	2.126	6	NS	0.091	6	NS
Leaf area (m ²)	0.405	6	NS	2.453	6	NS	0.171	6	NS

NS = >.05, * = <.05, ** = <.01, *** = <.005

Branches of lower order had significantly greater LSC and k_s values than branches of higher order (t-value = -4.03, df = 6, $p < 0.01$; figure 10 and t-value = -3.36, df = 6, $p < 0.05$; figure 11 respectively). The differences in cross-sectional sapwood areas between the two branching orders were not significant (t-value = 0.40, df = 6, $p > 0.05$; table 3). Similarly, there were no significant differences in leaf area or hydraulic conductivity (t-value = 0.40, df = 6, $p > 0.05$ and t-value = -0.05, df = 6, $p > 0.5$ respectively; table 3).

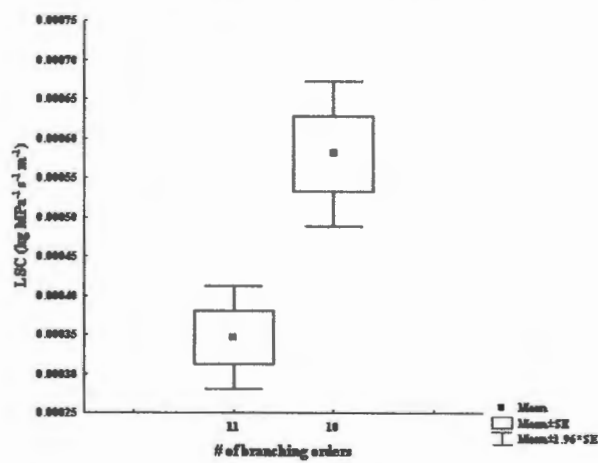


Figure 10: The leaf specific conductivities (LSC) measured at the 10th and 11th branching orders of *L. laureolum* individuals (t-value = -4.03, df = 6, N = 8, $p < 0.05$). Note: all measurements were taken the same number of nodes back from the branch tips on all individuals.

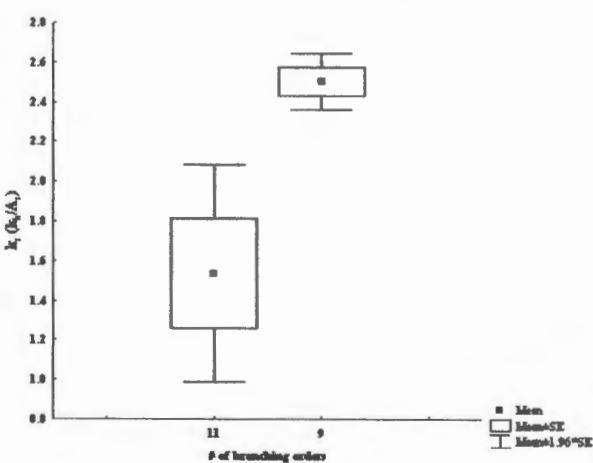


Figure 11: The specific conductivities (k_s) measured at the 10th and 11th branching orders on *L. laureolum* individuals (t-value = -3.36, df = 6, N = 8, $p < 0.05$). Note: all measurements were taken the same number of nodes back from the branch tips on all individuals.

Plants with greater numbers of branching orders had lower transpiration rates than plants with fewer branching orders (figure 12). Not all comparisons over one order of branching were significantly different ($p > 0.05$), but all comparisons over two orders were ($p < 0.05$; table 4).

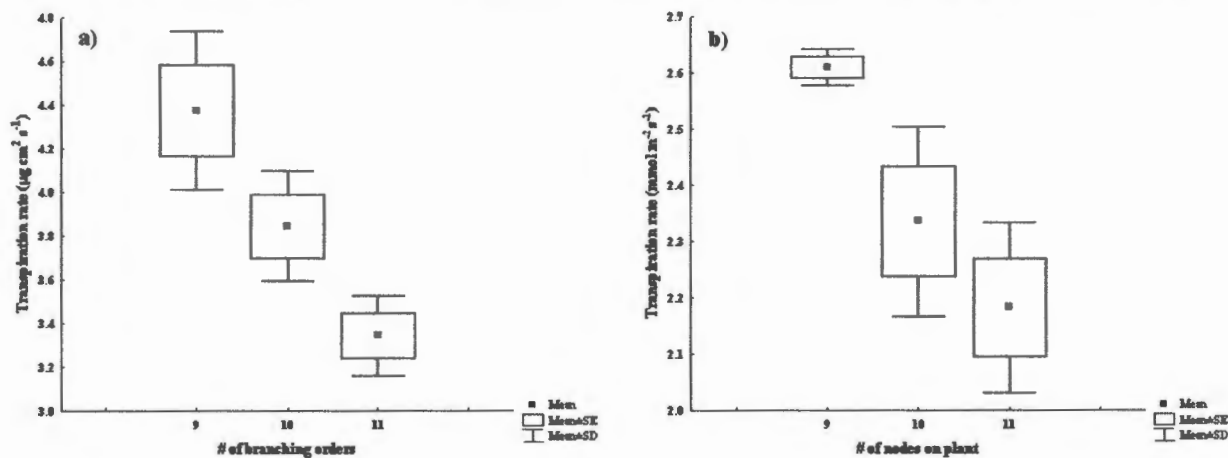


Figure 12: Transpiration rates measured in *L. laureolum* individuals with different numbers of branching orders. Transpiration rates were measured in (a) using a Li-Cor nul balance Steady-state porometer LI-1600 in $\mu\text{g cm}^{-2} \text{s}^{-1}$, and in (b) using an IRGA ADC LCA4 in $\text{mmol cm}^{-2} \text{s}^{-1}$. Each category consists of the means of three readings for each of three plants. Mann-Whitney U-test results shown in table 4.

Table 4: Mann-Whitney U-tests between transpiration rate readings for plants with 9, 10 and 11 branching orders each. Readings were taken using two independent apparatus, (a) a Li-Cor Porometer LI-1600, and (b) an IRGA ADC LCA4. Each category consists of the mean of three readings for three plants each.

	U	Z	p	Valid N group 1	Valid N group 2
a) Porometer					
9 vs 10	1.000000	-1.52753	NS	3	3
10 vs 11	0.000000	-1.96396	*	3	3
9 vs 11	0.000000	-1.96396	*	3	3
b) IRGA					
9 vs 10	0.000000	-1.96396	*	3	3
10 vs 11	2.000000	-1.09109	NS	3	3
9 vs 11	0.000000	-1.96396	*	3	3

NS = >.05, * = <.05, ** = <.01, *** = <.005

4) DISCUSSION

Hydraulic limitation as the result of accumulated branch nodal resistance was proposed as a possible mechanism explaining the trade-off between ramification and longevity in species of the Cape Proteaceae. In order to prove this hypothesis the aims of this study were to show that nodes are regions of greater resistance to xylem sap flow; to quantify the resistance imposed per node, and for a plant as a whole, in order to show that accumulated nodal resistance throughout a plant can be

severe; and to determine if there is any evidence for hydraulic limitation and compensation in the study species'.

a) Xylem vessel lengths

The combination of the study species having very short internodes, and the sampling design required to test the hypothesis, meant that hydraulic conductivity had to be measured on stem segments shorter than the longest xylem vessel lengths. As this is generally thought to result in unrealistically high conductivity readings that are not indicative of the norm (Sperry *et al.* 1988), the use of shorter segments requires justification.

The near direct relationships between the hydraulic resistances of the uncut segments and the sums of the hydraulic resistances of the component segments (figure 3; table 1) meant that vessel ends had very little effect on hydraulic conductance in *L. laureolum*. This could mean that the resistance of the vessel ends is small when compared to the resistance of the vessel lumen. If vessel endings had a large affect on conductivity measurements we would expect the resistance of the full segment to be much greater than the sum of the resistances of the individual segments.

The slopes of the regression lines for some of the individual nodes implied that the resistance of the summed component segments was slightly greater than that of the uncut segment (table1). It is possible that this is the result of non-linear flow at the segment ends (Chiu and Ewers 1993). The resistance of more segment ends would be included when calculating the sum of the resistances of the component segments than when measuring the resistance of the uncut segments. The amount of resistance created by the vessel ends is uncertain, but Chiu and Ewers (1993) found that it only affected conductivity measurements significantly in segments 1cm long.

The relationship between the resistances of the uncut segments and the sums of the resistances of the component segments was very variable in the *L. oleifolium* samples. The reason for this was unclear. The relationship did, however, remain strongly linear in all samples. This implies that the relative resistance of each component of the uncut segment stayed constant, and that the ratios used to test for nodal resistance should be little affected.

b) Identifying and quantifying hydraulic constriction in nodes

The differences in conductivity between the nodal segment (c) and the first basal segment (b) were significantly greater than the differences in conductivity between the first basal (b) and the second basal (a) ($p < 0.05$), or between the distal segments (d) and the nodal segment (c) ($p < 0.05$), for both species (table 2). This indicates that nodes are regions of greater hydraulic resistance in these two species.

The apparent increase in conductivity from the second basal (d) to the first basal segment (c) in a number of the samples (figure 4) could have resulted because the second basal segment was often cut very near to a node lower on the branch. When they examined the anatomy of branch nodes, Lo Gullo *et al.* (1995) found that branch junction constrictions were the result of increased numbers of vessel endings and narrower vessel diameters in the nodes, when compared to internodes. It is possible that the narrowing of vessels through the node result in a large number of vessel endings in the internodal region immediately distal to the node, thus creating greater resistance to sap flow.

The amount of resistance created by a node increased linearly with increased basal sapwood area of the node in *L. laureolum* ($R^2 = 0.82$, $p < 0.005$; figure 8). This relationship indicates that although the quantity of nodal resistance increases with increasing basal diameter of the node, the proportion of resistance created by each node, relative to the flow rate through the segment, stays constant. This is supported by the lack of a clear relationship between the proportion of a segment's resistance found in the node and the basal diameter of the node ($R^2 = 0.10$, $p > 0.5$; figure 9).

Each node creates roughly 70% of the resistance of a 5 cm segment in *L. laureolum*. Using the equation:

$$L_{\text{extra}} = [P_{\text{Rnode}} / (1 - P_{\text{Rnode}})] * L_{\text{segment}} \quad (3)$$

this resistance could be expressed as approximately 12 cm extra branch length of the same diameter as the base of each node (segment c; figure 2). Where L_{extra} = equivalent extra branch length, P_{Rnode} = proportion of the total resistance of the segment created by node, and L_{segment} = the length of the segment. Similarly in *L. oleifolium* approximately 80% of the resistance in a 4 cm segment could also be expressed as 16 cm extra branch length of the same diameter as the base of the node. As the number of nodes the plant has increases roughly exponentially with branching

order, the amount of extra resistance created by the nodes, or extra branch length that the nodes represent, should become substantial very quickly.

A model to determine the total extra branch length, equivalent to the nodal hydraulic resistance experienced by a plant, can be developed using the equation:

$$\text{Total } L_{\text{extra}} = \# \text{ of nodes on the plant} * L_{\text{extra}} \text{ (eq. 3)} \tag{4}$$

It may be necessary to estimate the number of nodes on the plant, as there are often a large number. This can be done using the equation:

$$\# \text{ of nodes on plant} = (RI)^0 + (RI)^1 + (RI)^2 + \dots \dots + (RI)^{\text{branching order} - 1} \tag{5}$$

where RI = the ramification index acquired by averaging the number of branches arising from each node for a random sub-sample of nodes.

The predicted equivalent extra branch lengths over seven orders of branching, for *L. laureolum*, using ramification indices of 1.5, 2.5 and 3.5 branches per node, and letting each node equal 12 cm extra branch length are shown in figure 13. The models with higher ramification indices accumulated equivalent extra branch length much faster than the models with lower ramification indices. The actual measured ramification index for *L. laureolum* was closest to 2.5.

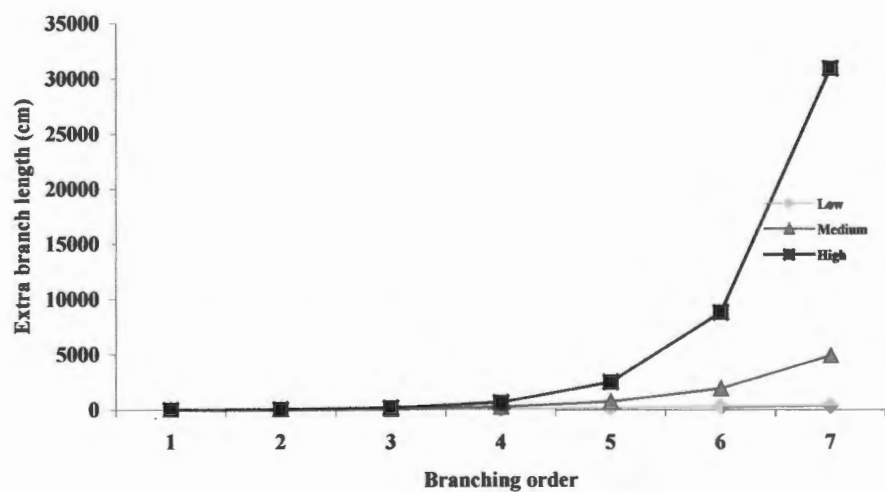


Figure 13: The predicted extra branch length, equivalent to nodal resistance, with increased branching order in *L. laureolum*, modelled using the product of equations (3) and (5). Low, medium and high refer to ramification indices of 1.5, 2.5 and 3.5 respectively.

Modeling the actual extra resistance, or drop in flow rate, caused by the nodes on a plant is more difficult. It requires knowledge about the number of branching orders in the plant, the number of nodes and the mean basal sapwood area of the nodes in each branching order, and the amount of resistance created by each node of particular basal sapwood area. The number of nodes for a

particular branching order can be calculated using a single term of equation (5) where the exponent is the branching order minus one. Given the basal sapwood area of the most basal node, and the number of nodes per branching order, a rough estimate of the mean basal sapwood area for each order of branching can be calculated using Da Vinci's law. Da Vinci's law states that the sum of the cross sectional areas of branches distal to a node is equal to the cross sectional area of the branch basal to the node (Tyree and Zimmermann 2002). Thus the equation:

$$\text{Mean basal area of nodes in branching order } y = \text{Basal area of most basal node} / \text{Estimated number of nodes in branching order } y \quad (6)$$

gives an estimate of the mean cross sectional area of the base of each node for a given branching order. The amount of resistance created by nodes with different basal sapwood areas can be calculated by substituting the basal sapwood area into the regression equation for a scatter-plot of the amount of resistance created against the basal sapwood area of the node, similar to those shown in figure 8.

Thus the total resistance created by each branching order can be calculated using the equation:

$$R_{BOx} = (RI)^{bo-1} * (a * (\text{basal area of the basal node} / ((RI)^{bo-1})) + c) \quad (7)$$

using a linear regression equation, or:

$$R_{BOx} = (RI)^{bo-1} * (a * \ln(\text{basal area of the basal node} / ((RI)^{bo-1})) + c) \quad (8)$$

using a logarithmic regression equation,

where R_{BOx} = the total resistance of branching order x, RI = ramification index, bo = branching order, a = the slope and c = the y-intercept of the regression equation. The total resistance created by nodes in the whole plant can be calculated by summing the total resistance of each branching order.

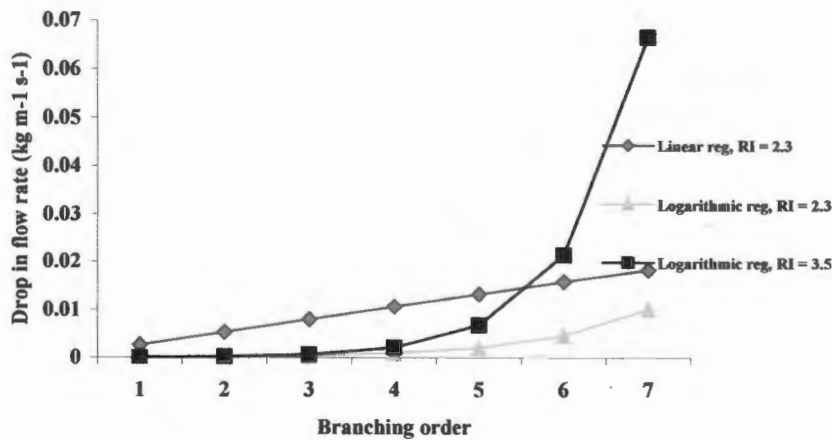


Figure 14: The predicted drop in flow rate caused by nodal resistance with increased branching order in *L. laureolum*. Modelled using equations (7) and (8) and the regression equations given in figure 8. The drop in flow rate modelled using the linear regression equation is not influenced by changes in the ramification index (RI). The models using the logarithmic equation are influenced by changes in the RI.

The predicted drop in flow rates caused by nodes over seven orders of branching for *L. laureolum*, using the model and the regression equations given in figure 8, and ramification indices of 2.3 and 3.5 are shown in figure 14. The actual measured ramification index for *L. laureolum* was closest to 2.3. When the linear regression equation was used to predict the drop in flow rate caused by nodes of different basal sapwood area, the model (the sum of the total resistance of each branching order, given by equation (7)) predicted a linear increase in total nodal resistance with increased branching order. The resistance predicted by this model was not influenced by the ramification index. This was because as the number of nodes in the branching order increased the mean basal sapwood area of each node, and thus the amount of resistance created by each node, decreased by the same proportion. This implies that although a more ramified plant accumulates equivalent extra branch length much faster than a less ramified plant, the actual amount of hydraulic resistance created by nodes in each plant is the same. This is because the nodes in the more ramified plant have smaller basal sapwood areas, and thus create less resistance per node, than the nodes in the less ramified plant. If the relationship between basal sapwood area and the drop in flow rate caused by a node is truly linear (figure 8), then two equal aged plants can only differ in terms of their total nodal resistance if one has more orders of branching than the other. This can occur if one plant begins branching earlier in its lifetime than the other, which is the case in more ramified Cape Proteaceae (Bond and Midgley 1995), or if one plant branches more often than the other (i.e. more than once a year in the Cape Proteaceae). If nodes that give rise to more distal branches create greater resistance to sap flow than nodes that give rise to fewer distal branches, then differences in the degree of ramification of two equal aged plants, with the same number of branching orders, could create differences in the amount of total nodal resistance suffered by each plant. Unfortunately sample sizes in this study were too small to test for such differences statistically. Further study is required to investigate these factors within, and between, species. When the logarithmic equation (eq.8) was used in the model the total nodal resistance increased exponentially with increased branching order. The increase in total resistance was predicted to be much faster in more ramified plants. Should more intensive sampling show that the true relationship between basal sapwood area and the drop in sap flow caused by the node is actually logarithmic, then it would provide even stronger evidence that more ramified plants have shorter life-spans than less ramified plants because they accumulate nodal hydraulic resistance faster. The nodes sampled for figure 8 were all within two branching orders of each other and ranged in basal diameter from only 3.2 to 8.6 mm. It is thus quite likely that the relationship shown in figure 8 will change with more intensive sampling.

c) Possible effects of hydraulic limitation, and mechanisms of compensation, in different sized *L. laureolum* individuals

Greater specific conductivity values in the lower order branches of *L. laureolum* individuals meant that they imposed less resistance to water flow per unit sapwood area than the higher order branches. This implies that the lower order branches had, on average, larger xylem vessel diameters than the higher order branches. This would result from hydraulic limitation on xylem vessel development. Xylem vessel diameter has been found to increase with increased available water during the period of xylem development (Carlquist 1975). This has been reported for two species in the Cape Proteaceae (February and Manders 1999). The greater resistance to sap flow in the higher order branches, caused by the extra node and branch length, would have limited the amount of water available to the area of xylem development, thus limiting the vessels to smaller diameters, less vulnerable to embolism (Sperry and Tyree 1988). Hubbard *et al.* (1999) found that increased effects of gravity reduced the specific conductivity of branches. It is unlikely that gravity played a significant role in the differences found in this study as Hubbard *et al.* (1999) found that it accounted for only 10 – 20 % of the difference between Ponderosa pines of 10 and 30 metres. The difference in length between *L. laureolum* branches that differed by one branching order was only 10 – 15 cm.

Leaf specific conductivity was significantly lower in higher order branches of *L. laureolum* individuals ($p < 0.05$; figure 10). Ewers and Zimmermann (1984) found that higher order branches had lower leaf specific conductivities than lower order branches too. They, and Tyree *et al.* (1991), suggested that this was because higher order branches had narrower stems, and leaf specific conductivities decrease with decreasing stem diameters because xylem vessel diameters, and thus specific conductivities, decrease accordingly. The differences in sapwood area between the lower and higher order branches in *L. laureolum* individuals were not significant (table 3), and thus could not be responsible for the significant difference in specific conductivity ($p < 0.05$; figure 11), or leaf specific conductivity. The lack of significant difference in hydraulic conductivity and leaf area between branches of the two orders (table 3) implies that these factors did not bias the results either. Lower leaf specific conductivities in higher order branches are thus likely to be the result of narrower xylem vessels due to hydraulic limitation on development, rather than due to narrower stem diameters.

Transpiration rates were demonstrated to be lower in plants with higher branching orders using two independent apparatus (figure 12; table 4). As transpiration rate is a measure of gas

exchange it can also be interpreted as a measure of carbon gain (Tyree and Zimmermann 2002). Plants with higher branching orders are thus subject to lower rates of photosynthesis per unit leaf area. The lower transpiration, and thus photosynthetic, rates would have been limited by the lower leaf specific conductivity demonstrated in higher order branches. Hydraulic resistance, or low leaf specific conductivity, causes a reduction in leaf water potentials (Tyree and Zimmermann 2002), and stomata close when leaf water potential reaches a critical level beyond which the water column will cavitate (Sperry and Tyree 1988). Hydraulic resistance to sap flow thus limits rates of photosynthesis in *L. laureolum* individuals. That hydraulic conductance limits photosynthesis in *L. laureolum* is consistent with the hypothesis of hydraulic limitation, and findings, of Hubbard *et al.* (1999). Hubbard *et al.* (1999) provided direct proof that rates of transpiration, and thus photosynthesis, in *Pinus ponderosa* individuals are determined by leaf specific conductivity by performing leaf removal experiments. Transpiration rates increased when leaves were removed (i.e. when leaf specific conductivity was increased) indicating that transpiration rates were hydraulically limited.

The mean leaf specific conductivity of the lower order branches was nearly twice as much as the higher order branches (figure 10). The differences in transpiration rates between the two branching orders were far smaller (figure 12). For the higher order branches to maintain similar transpiration rates to the lower order branches their water columns were most likely under much greater stress, and thus more vulnerable to cavitation (Sperry and Tyree 1988). This demonstrates the increased risk of fatal embolism in *L. laureolum* individuals that suffer greater hydraulic stress as the result of increased nodal resistance. There was, however, no significant reduction in leaf to sapwood area ratios in higher order branches of *L. laureolum* individuals. Such a reduction has been reported in a number of studies (Hubbard *et al.* 2002, McDowell *et al.* 2002a & b), and has been suggested as a mechanism to compensate for hydraulic limitation. Lower leaf to sapwood area ratios means that there is more sapwood supplying each unit of leaf area, allowing leaves to maintain high rates of transpiration while not significantly increasing the probability of stomatal closure or embolism through increased stress on the xylem vessels (Hubbard *et al.* 2002). Differences in leaf to sapwood area ratios may not have been evident because comparisons were made over only one order of branching. McDowell *et al.* (2002a) and Hubbard *et al.* (2002) reported only small differences in leaf to sapwood area ratios in trees that differed in height by up to 60 metres.

d) Conclusions

This study has highlighted the importance of branch junction constriction and hydraulic limitation in limiting size and/or age of individuals in the Cape Proteaceae. Results indicate that branch nodes cause significant reduction in sap flow rates in *Leucadendron laurum* and *Leucospermum oleifolium*. Data from models indicate that whole plant resistance to sap flow increases rapidly with increased branching order. This implies that plants that begin branching earlier, and/or branch with greater temporal frequency, become subject to greater hydraulic resistance earlier in their lifespan, and thus may be limited to younger maximum age.

Transpiration rates, branch specific (k_s), and leaf specific (LSC) conductivities were found to be significantly lower in higher order branches of *L. laurum*. Specific conductivities may have been lower in higher order branches as greater nodal resistance to sap flow would limit the amount of water available to the area of developing xylem, thus causing the vessels to have narrower diameters. There was no evidence of hydraulic compensation, as reported elsewhere, as higher order branches did not have reduced leaf area despite having lower specific conductivities. As the specific conductivities (k_s) were lower in higher order branches, but the leaf area did not decrease accordingly, the leaf specific conductivities (LSC) were also much lower. The low leaf specific conductivities caused transpiration rates to be lower in higher order branches. As transpiration rates are a good indication of xylem conductivity this further suggests increased hydraulic limitation on plant production with increased branching order. Hydraulic resistance in nodes thus imposes an important limitation on size and/or age of *L. laurum* individuals.

This study suggests that hydraulic limitation as the result of accumulated branch junction constriction characterizes the trade-off between ramification and longevity. It is important to bear in mind that this is a trade-off and early senescence is not necessarily detrimental to the persistence of more ramified Proteaceae. Natural selection dictates that species should not evolve traits that are detrimental to their persistence. Species that are more ramified, and thus have shorter life spans, are adapted to environments with short fire return periods and most likely fare better in these environments than less ramified, longer lived species.

5) ACKNOWLEDGEMENTS

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